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Elevation in the Sucrose Content of the Shoot Apical Meristem of *Sinapis alba* at Floral Evocation[1]

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ABSTRACT

Nanogram tissue samples from apical meristems of *Sinapis alba* were assayed for sucrose, total soluble hexosyl equivalents (≡ glucose and fructose plus hexoses from sucrose hydrolysis), and total soluble glucosyl equivalents (≡ glucose plus glucose from sucrose hydrolysis). On dry weight basis, sucrose concentration increased by more than 50% within 10 hours after the start of either a long photoperiod or a short photoperiod displaced by 10 hours in the 24-hour cycle ('displaced short day'). (These treatments induce flower initiation) Glucose and fructose concentrations were close to zero in vegetative meristems and remained low compared to sucrose in meristems of induced plants. Within a single meristem, the peripheral and the central zones had similar concentrations of sucrose. Our results indicate that an early physiological event in floral transition is the accumulation of sucrose in the meristem.

The transition of a vegetative meristem to a floral meristem is marked by several histological, cellular, physiological, and molecular changes (2); those events that are associated with an irreversible commitment of the meristem to initiate flowers constitute the 'evocation' (12). One physiological change that is associated with floral transition in photoperiodic- and cold-requiring plants is an increase of the soluble carbohydrate concentration in the apical bud (7). In *Sinapis alba*, this increase has been observed regardless of the environmental inductive treatment (3, 4) and is not related to an increase of the photosynthetic assimilation rate (M. Bodson, unpublished data). Significant increases have been detected within 10 to 14 h after the start of an inductive treatment and, thus, this change has been hypothesized as a critical event for the evocation of the meristem of *S. alba* (3, 4). However, these observations were based on gross tissue samples, including leaf primordia, stem, and the meristem *per se*. As evocation is probably restricted to the meristem itself, a more definitive approach is to determine changes occurring only in the meristem.

Within the meristem, there are three different histological regions: (a) the central zone at the summit of the dome; (b) the peripheral zone—which is the site of organogenesis—on the flanks of the meristem; and (c) the pith-rib meristem located internally just underneath the central zone. In the vegetative meristem, the central zone is less active mitotically than the peripheral zone (13, 17, 19). At floral evocation, both the central and the peripheral zones are activated mitotically and the pith-rib meristem progressively vacuolates and disappears (2, 13).

At least in *Tradescantia paludosa*, the mitotic activity of the central zone is influenced by nutritional factors (27). Thus, one possibility is that the central zone of the vegetative meristem is relatively deprived of nutrients and that an increase of assimilates in it is a prerequisite for floral transition (23). However, there are no data in support of this hypothesis.

The highly sensitive methods for quantitative histochemical analysis described by Lowry and Passonneau (16, see also 20) are well suited to study the metabolite content of the meristem. Using these methods, we have measured the sucrose content of the peripheral zone and central zone of the apical meristem of *Sinapis* plants, which were induced to flower by one LD or by one displaced SD. Total soluble hexosyl equivalents and total soluble glucosyl equivalents were measured also, but with less morphological resolution.

MATERIALS AND METHODS

Plant Material. *Sinapis alba* plants were cultured as described elsewhere (3). Vegetative plants were maintained by growing them under 8-h photoperiods (SD). Sixty-five-d-old plants were induced to flower either by one 22-h LD or by one 8-h displaced SD (Fig. 1). Subsequently, the induced plants were returned to the standard SD regime. The meristem of induced plants will be referred to as 'evoked' to signify that the meristem of these plants is undergoing evocational events. Control plants were maintained continuously under the standard SD conditions. Shoot tips of control and LD-induced plants were collected 10 and 16 h after the start of the LD. Shoot tips of control and displaced SD-induced plants were collected 8 h after the start of the displaced SD.

Preparation of Samples. Shoot tips were frozen quickly in liquid N_2 slurry and then transferred to Dry Ice. Then, the

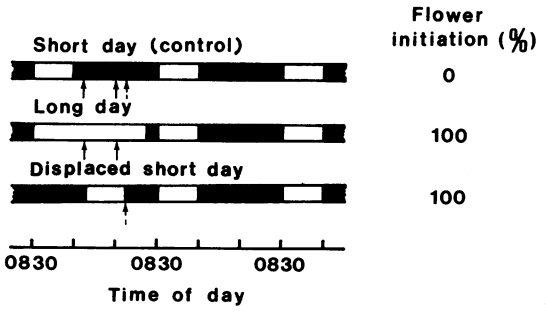

FIG. 1. Flower initiation in *S. alba* in response to different photoperiodic cycles. Light irradiance: 160 $\mu E \cdot m^{-2} \cdot s^{-1}$. Solid bars represent darkness. Solid arrows represent sampling times for LD-induced plants, broken arrows for displaced SD-induced plants.

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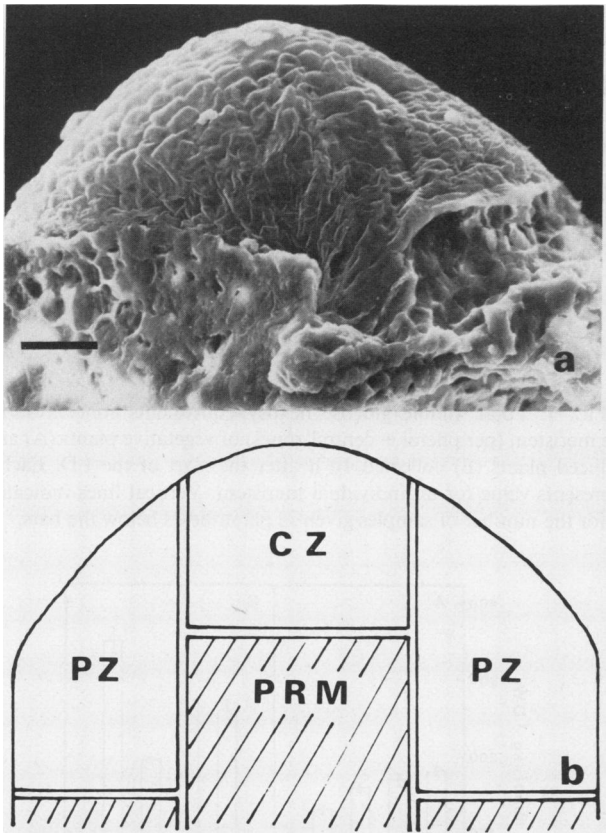

FIG. 2. a, Scanning electron micrograph of *S. alba* meristem isolated from a freeze-dried apical bud. Magnification line = 20 μm. b, Longitudinal section of the meristem showing regions that were isolated by dissection. The peripheral zone (PZ) and the central (CZ) were analyzed. PRM, pith-rib meristem.

samples were freeze-dried (−40°C, ~10 μm Hg, 72 h). The dried samples were stored at −20°C under vacuum. One or more samples (15–25 ng) of the peripheral zone and/or central zone of the apical meristem (Fig. 2) were dissected and weighed on a fishpole balance (16). These procedures were carried out under ambient conditions of 19°C and 40% RH. (Preliminary experiments had shown that sucrose in *Sinapis* tissue was stable during these procedures)

Biochemicals. Enzymes, except invertase, were from Boehringer; invertase and most other reagents were supplied by Sigma. To decrease ($[NH_4]_2SO_4$) in the final reagents, enzymes supplied as suspensions were centrifuged, the supernatant was discarded, and the pellet was dissolved in 25 mM Tris-HCl (pH 8.1) containing 0.02% (w/v) BSA. (When diluted in the working solution, the concentration of Tris was insufficient to dilute invertase) Enzyme solutions were stored at 4°C and prepared fresh weekly. All calculations were based on enzymically standardized stock reagents.

Assays. Sucrose was assayed by the procedure of Jones *et al.* (15) except DTT was omitted and the specific step reagent was amended to include 0.5 mM EDTA. (These reagent changes were made as a result of preliminary experiments with $HClO_4$ extracts of fresh *Sinapis* tissue)

Total soluble glucosyl equivalents (glucose plus glucose from sucrose hydrolysis) and total soluble hexosyl equivalents (glucose and fructose plus hexoses from sucrose hydrolysis) were assayed by the procedure of Outlaw and Manchester (21).

Preliminary experiments to validate the assays with *Sinapis* tissue were conducted with $HClO_4$ extracts of young leaf samples. In brief, the kinetics of the specific step reaction confirmed specificity, stability of the indicator, and linearity with tissue mass.

Statistical Analysis. Measurements were performed on 3 to 5 individual meristems and, for each meristem, 8 to 19 samples were examined. SE is given for the mean values of individual meristems (graphs 3–9) and of pooled values (Tables I and II). Additionally, statistical analysis of the pooled values was performed using the two-way analysis of variance based on the hierarchical model.

RESULTS

At 10 h (Fig. 3) after the beginning of the LD, sucrose concentrations in the peripheral and central zones of the evoked meristems were, respectively, 155.5 ± 4.1 mmol·kg^{-1} DW[3] (n = 33) and 154.5 ± 5.8 mmol·kg^{-1} DW (n = 14), while the sucrose concentrations in the control meristems were 93.7 ± 4.2 mmol·kg^{-1} DW (n = 32) in the peripheral zone and 109.1 ± 10.0 mmol·kg^{-1} DW (n = 9) in the central zone. Similar observations were made 16 h after the start of the LD when sucrose concentrations in the peripheral and central zones of evoked meristems were, respectively, 151.8 ± 5.2 mmol·kg^{-1} DW (n = 53) and 151.8 ± 8.9 mmol·kg^{-1} DW (n = 24) as compared to 106.2 ± 3.8 mmol·kg^{-1} DW (n = 47) and 104.3 ± 9.0 mmol·kg^{-1} DW (n = 20) for control meristems (Fig. 4). Statistical analysis of the pooled values for the central and peripheral zones showed that the sucrose concentration of the evoked meristems was higher than that of control meristems at the 5% level of significance. Similarly, the sucrose concentrations of the peripheral and central zones of the meristem of displaced SD-induced plants were higher than those in the control plants (129.6 ± 2.6 mmol·kg^{-1} DW [n = 49] *versus* 84.5 ± 2.5 mmol·kg^{-1} DW [n = 49] for the peripheral zone and 140.8 ± 6.2 mmol·kg^{-1} DW [n = 17] *versus* 89.0 ± 4.0 mmol·kg^{-1} DW [n = 18] for the central zone, Fig. 5). Regardless of treatment, the sucrose concentrations in the peripheral and the central zones within a single meristem were similar (Figs. 3–5).

Hexosyl-equivalents were assayed in the meristem, but the peripheral and central zones were not assayed separately. By 10 h after the start of the LD, the total soluble hexosyl-equivalents concentration in the meristem was 349.5 ± 15.7 mmol·kg^{-1} DW (n = 24), compared to 154.6 ± 6.3 mmol·kg^{-1} DW (n = 24) for control plants (Fig. 6) while total soluble glucosyl-equivalents concentrations for LD and control plants were, respectively, 197.8 ± 8.3 (n = 24) and 91.6 ± 4.6 mmol·kg^{-1} DW (n = 24)

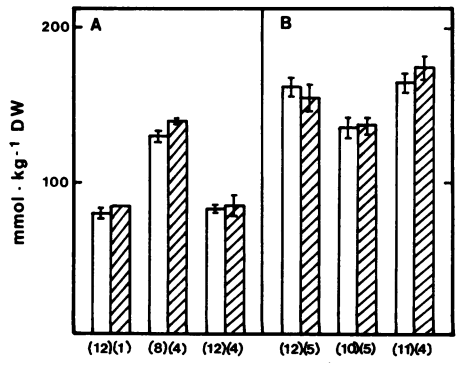

FIG. 3. Sucrose concentration in the peripheral (open bars) and the central (hatched bars) zones of the meristem of vegetative plants (A) and of induced plants (B) collected 10 h after the start of the LD. Each pair of bars represents values for an individual meristem. Vertical lines indicate the SE for the number of samples given in parentheses below the bars.

[3] Abbreviation: DW, dry weight.

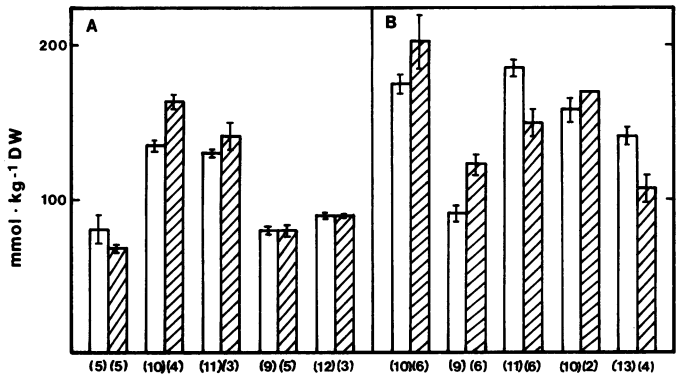

FIG. 4. Sucrose concentration in the peripheral (open bars) and the central (hatched bars) zones of the meristem of vegetative plants (A) and of induced plants (B) collected 16 h after the start of the LD. Each pair of bars represents values for an individual meristem. Vertical lines indicate the SE for the number of samples given in parentheses below the bars.

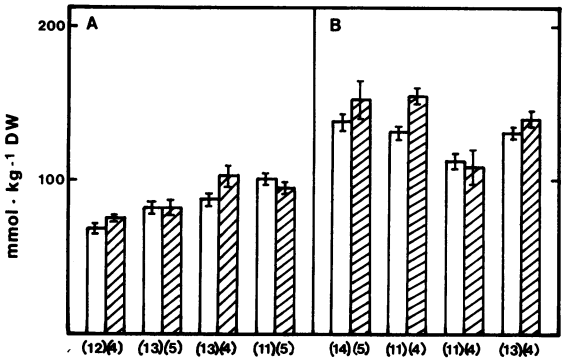

FIG. 5. Sucrose concentration in the peripheral (open bars) and the central (hatched bars) zones of the meristem of vegetative plants (A) and of induced plants (B) collected 8 h after the start of the displaced SD. Each pair of bars represents values for an individual meristem. Vertical lines indicate the SE for the number of samples given in parentheses below the bars.

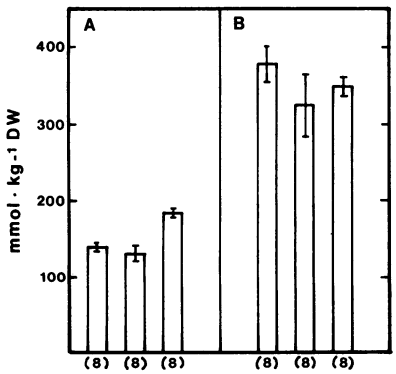

FIG. 6. Total soluble hexosyl-equivalents concentration in the meristem (peripheral + central zones) of vegetative plants (A) and of induced plants (B) collected 10 h after the start of the LD. Each bar represents value for an individual meristem. Vertical lines indicate the SE for the number of samples given in parentheses below the bars.

(Fig. 7). Similar assays of meristem from plants induced by a displaced SD confirmed the increase in both total soluble hexosyl- [284.8 ± 13.2 (n = 37) *versus* 164.3 ± 4.6 mmol·kg^{-1} DW (n = 30), Fig. 8] and total soluble glucosyl-equivalents concentrations (144.4 ± 6.7 [n = 33] *versus* 81.3 ± 2.4 mmol·kg^{-1} DW

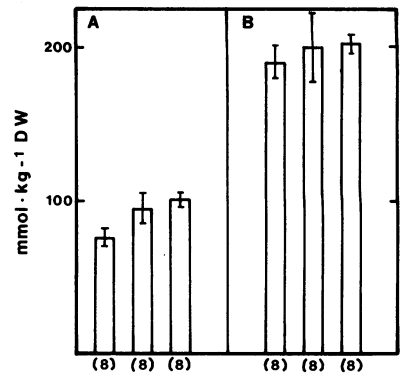

FIG. 7. Total soluble glucose hexosyl-equivalents concentration in the meristem (peripheral + central zones) of vegetative plants (A) and of induced plants (B) collected 10 h after the start of the LD. Each bar represents value for an individual meristem. Vertical lines indicate the SE for the number of samples given in parentheses below the bars.

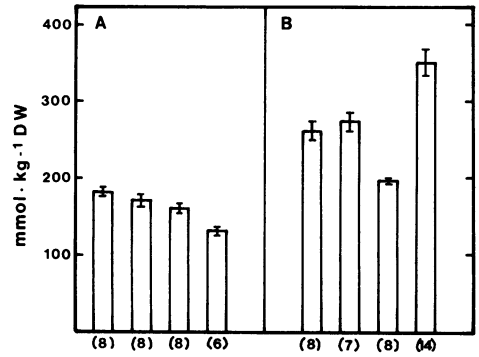

FIG. 8. Total soluble hexosyl-equivalents concentration in the meristem (peripheral + central zones) of vegetative plants (A) and of induced plants (B) collected 8 h after the start of the displaced SD. Each bar represents value for an individual meristem. Vertical lines indicate the SE for the number of samples given in parentheses below the bars.

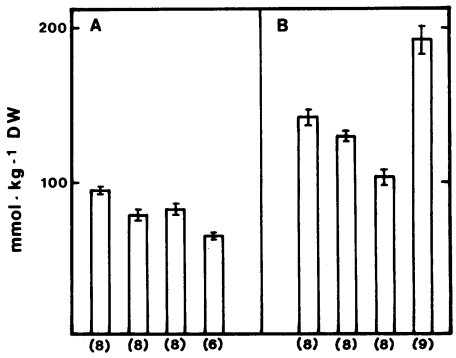

FIG. 9. Total soluble glucosyl-equivalents concentration in the meristem (peripheral + central zones) of vegetative plants (A) and of induced plants (B) collected 8 h after the start of the displaced SD. Each bar represents value for an individual meristem. Vertical lines indicate the SE for the number of samples given in parentheses below the bars.

[n = 30], Fig. 9).

Tables I and II summarize the changes in the soluble carbohydrate concentration in the meristem of plants induced to flower by a LD (Table I) and by a displaced SD (Table II). Regardless of the inductive treatment, sucrose concentration increased in evoked meristem by more than 50% of the control values. In evoked meristems, there was also an increase in the

Table I. *Soluble Carbohydrate Concentration in the Meristem (Peripheral + Central Zones) of Sinapis alba Induced to Flower by a LD*

Samples were collected 10 h after the beginning of the LD.

Carbohydrate	Control	Evoked	Difference	Increase
	mmol·kg⁻¹ DW; $\bar{X} \pm SE$; n			%
Sucrose[a]	97.1 ± 4.0; 41	155.2 ± 3.3; 41	58.1 ± 5.2	59.8
Total soluble hexosyl equivalents[a]	154.6 ± 6.3; 24	349.5 ± 15.7; 24	194.6 ± 16.9	125.8
Total soluble glucosyl equivalents[a]	91.6 ± 4.6; 24	197.8 ± 8.3; 24	106.2 ± 9.5	115.9
Glucose (calculated)	−5.5 ± 6.3	42.6 ± 7.5	48.1	
Fructose (calculated)	−34.2 ± 6.6	−3.2 ± 11.6	−31	

[a] Difference between control and evoked significant at $P \leq 0.05$.

Table II. *Soluble Carbohydrate Concentration in the Meristem (Peripheral + Central Zones) of Sinapis alba Induced to Flower by a Displaced SD*

Samples collected 8 h after the beginning of the displaced SD.

Carbohydrate	Control	Evoked	Difference	Increase
	mmol·kg⁻¹ DW; $\bar{X} \pm SE$; n			%
Sucrose[a]	85.7 ± 2.1; 67	132.5 ± 2.6; 66	46.8 ± 3.3	54.6
Total soluble hexosyl equivalents[a]	164.3 ± 4.6; 30	284.8 ± 13.2; 37	120.5 ± 15.3	73.3
Total soluble glucosyl equivalents[a]	81.3 ± 2.4; 30	144.4 ± 6.7; 33	63.1 ± 7.4	77.6
Glucose (calculated)	−4.4 ± 3.5	11.9 ± 6.0	16.3	
Fructose (calculated)	−2.6 ± 3.7	5.9 ± 8.2	8.5	

[a] Difference between control and evoked significant at $P \leq 0.05$.

total soluble hesoxyl- and total soluble glucosyl-equivalents concentrations but both increases were higher for LD-induced plants (~120% of the control values) than for displaced SD-induced plants (~75% of the control values).

In control meristems, calculated glucose and fructose concentrations were close to zero (Tables I and II). Even in evoked meristems, the hexose concentration (glucose or fructose) remained low compared to sucrose (less than 15% on an anhydrohexosyl basis). Although the concentration of glucose appeared to increase in one set of experiments (LD-induced plants), we do not consider the change to have been unequivocally demonstrated. The values for glucose and fructose are calculated from the measurements of sucrose alone, glucosyl equivalents, and hexosyl equivalents. Thus, when the sucrose concentration is relatively high as in these experiments, substantial errors in the derived data may accrue.

DISCUSSION

Our results show that: (a) the sucrose concentration of the meristem increases at floral evocation; and (b) the absolute concentrations in the different zones are similar within any one meristem, which indicates that the zonation pattern of the meristem is not related to differences in sucrose concentration in the individual zones. Other reports (9, 10) indicate that neither fructose 6-P kinase nor glucose 6-P dehydrogenase is assymetrically distributed within the vegetative meristem of *Dianthus*. On the basis of these observations, we suggest that factors other than those related to the soluble carbohydrate metabolism are responsible for meristem zonation.

We have also shown here that glucose and fructose concentrations are very low compared to sucrose. These calculated values are higher if the meristem is undergoing floral evocation. However, these derived values for the hexoses are inexact and must be interpreted with caution because: (a) they were calculated from measurements made on different tissue samples (technical reasons that prevent sucrose and soluble hexosyl determinations within the same sample have been discussed elsewhere [21]); and (b) among individual meristems, there is some variability of the sucrose or soluble hexosyl concentrations, *e.g.* of 26 evoked meristems analyzed for either sucrose or soluble hexosyls, five were found to have concentrations similar to those of vegetative meristems.

The correlation between sucrose concentration in the meristem and floral evocation is of particular interest. Several studies have emphasized the promotive effect of sucrose on flower initiation of intact plants (7) or with plant material grown *in vitro* (8, 11, 18). The present study is the first to show unequivocally that the sucrose concentration increases in the meristem of plants induced to flower. (This possibility was raised in an earlier report on whole apical buds [3]) With both inductive treatments, the increase of the soluble carbohydrate content occurs at a very early time of the floral transition, as soon as 8 to 10 h after the start of induction and well before any morphological changes can be detected in the meristem (2, 6).

Sucrose is a ubiquitous compound and serves diverse roles in a plant's physiology. In the present context, two possible explanations for the relationship between floral evocation and sucrose accumulation in *Sinapis* meristem shall be discussed. First, it is possible that sucrose accumulation may decrease the solute potential and, thus, provide the driving force for cell expansion occurring at evocation (14). However, this hypothesis fails when examined quantitatively: we estimate that the bulk solute potential would be decreased by less than 0.25 bar by the accumulated sucrose. Even if sucrose were restricted solely to the cytoplasm, it does not appear that the change in osmolarity would be significant. Second, there is evidence that in the meristematic zone of roots, carbohydrate availability controls the cell's progression through the mitotic cycle (26). At evocation, there is a stimulation of the proliferation of the shoot meristem cells (2) which is only partly controlled by cytokinin (1). Thus, sucrose accumulation may be necessary for the control of meristematic cell proliferation, providing additional substrate for respiration. Although ATP concentration increases in evoked buds (5), an explanation based only on respiration requirement fails; using respiration rates obtained with wheat buds (25) and the sucrose concentration reported here, we estimate that the sucrose turnover time in the meristem would be approximately 5 h. As the meristematic region is small and within centimeters of source leaves, sucrose redistribution to meet this demand would not

seem to be limiting. However, an argument based on sucrose availability *per se* neglects other important considerations. Regardless of whether the meristem is evoked, the hexose concentration was low compared to the concentration of sucrose. If the flux through the pathway from sucrose through glycolysis is elevated at evocation, the probability is that sucrose hydrolysis is rate limiting. The $S_{0.5}$ for sucrose synthetase (~50 mM) (24) and the K_m for invertase (~3–10 mM) (22) are in the range of our estimate for sucrose concentration in *Sinapis* meristem (10 and 17 mM for control and evoked meristems, respectively). Thus, the sucrose concentration increase upon evocation that we report could serve to speed sucrose hydrolysis. While we feel that this interpretation has merit, it should be validated by assays of the relevant enzymes and their effectors.

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